Research Activities

San Juan River Population Model Refinements.
Fiscal Year 2002 Project Proposal

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Background:

A modeling effort to construct a conceptual framework for the fish community and endangered fishes in the San Juan River began in 1998. This effort relates to Sections 5.1; 5.1.1; 5.1.2; 5.1.3.; 5.1.4 of the Long Range Plan. These models have helped direct a focused field effort with the intent of using key site specific data to determine the carrying capacity of pike minnows and razorback suckers in the river.

That models as proposed includes bioenergetics, population, and trophic components. Data for fish populations by age class and habitats as well as other trophic components are required as model parameters. The intent of the 2002 program is to better parameterize structural and functional components of these conceptual models. Three approaches are currently under investigation each of which is centered on a different hierarchical organization. They are:

A. BIOENERGETICS This approach is individual based (structural) and summed for population effects .In addition, functional energetic (ingestion/egestion, assimilation, etc.) Are considered.

B. POPULATIONS This conceptual approach utilizes densities/biomass, size/age structure, etc of individual populations. The populations sizes will be tiered to habitats and habitat requirements.

C. TROPHIC STRUCTURE An attempt is being made to understand the food web structure of the river based upon functional groups. This approach utilizes biomass estimates at all trophic levels and will look at the movement of energy and biomass between trophic groups (IE grazers, detritivores)

Population estimates collected in 1998, 1999, 2000 and those scheduled for 2001 will be used in model verification and validation. No field data collections are proposed for FY2002. The focus of FY2002 is to refine the model into a working framework that includes all three of the above

approaches into one model. In addition, new components may be added to the model to address new fish species not in the original model. These species include roundtail chub and striped bass.

Other tasks for FY2002 are preparation of the draft and final report, model documentation, and integration of 1998 through 2001 population estimate data.

This additional population effort is intended to provide additional information for use in refining the correlation between population estimates and relative abundance data.

Population dynamics of lotic fish communities are largely a function of the condition of and changes in their physical environment and the resulting responses in both primary (phytoplankton and periphyton) and secondary (zooplankton, micro-and macro invertebrates) production, and upon which these fishes rely to varying degrees for forage. Although the importance of these relationships are universally recognized by fisheries researchers, these lower trophic levels and the physical processes which influence them are often poorly understood in many aquatic systems. Yet these physical (substrate characteristics, temperature, water transparency, dissolved oxygen, etc.) and biological components of the ecosystem form the framework within which fish populations exist and function. In the San Juan and Animas Rivers, these factors are highly influenced by the flow regime associated with the annual spring runoff as well as summer storm events. This influence of the flow regime makes the study of these physical and biological components of the ecosystem especially relevant in rivers where the management of flow is considered vital to the health of t of fish population of concern.

Objectives:

1.) Further refine the correlation between population and abundance of fish using the first pass of population estimate and relative abundance during monitoring surveys.

2.) Incorporate data on forage base in the San Juan and Animas rivers for use on carrying capacity determinations in the population model.

3.) Determine the temporal changes in physical and biological conditions in selected riffle and run habitats in the San Juan and Animas Rivers in order to calibrate the bioenergetics-trophic model.

Methods:

The model will be produced using Stella software and the Wisconsin bioenergetics models. The modeling software will be configured to include multiple species and lifestages. Model documentation will be produced an Adobe PDF format for distribution.

Schedule:

Model refinement will be concurrent with preparation of model documentation. Model refinement will begin with the notice that funding has been secured. Completion of the model documentation is scheduled for 9 months after the start date.

Products:

A draft and final report on model documentation that includes a description of model application and use will be delivered to the Biology committee.

Budget FY-2002:

All funding for FY 2002 activities are requested from the recovery program. Total funding requested is shown in the following table.

	Miller Ecological Consultants	Ecosystems/ Keller-Bliesner	Total Cost
Labor	$ 19,150.00 (50 Staff days)	$ 12,530.00 (25 Staff days)	$ 31,680.00
Travel	$ 1,324.00 (10 staff days)	$ 2,100.00 (10 Staff days)	$ 3,424.00
Equipment	$ 1,110.00	$ 0.00	$ 1,110.00
Supplies	$ 0.00	$ 500.00	$ 500.00
Overhead	$ 0.00	$ 1,348.00	$ 1,348.00
Total	$ 21,584.00	$ 16,478.00	$ 38,062.00

Characterization of Razorback Spawning Bar
Fiscal Year 2002 Project Proposal
(2-year study)

Principal Investigator: Ron Bliesner
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Study Area:

The study area consists of the San Juan River in the Vicinity of Aneth, UT, or other identified Razorback Spawning locations.

Collections:

Interstitial and course substrate samples for grain size analysis.

Background:

Razorback sucker monitoring has identified aggregations of ripe males and females in a primary location in the river near Aneth, UT the last three years during typical spawning time. While actual spawning has not been observed, the conditions are right for spawning to occur and larval razorback suckers have been found in the larval drift studies downstream of this site. Since little is known about suitable spawning habitat for razorback sucker in the San Juan River, characterizing this site at or near the time of fish observation could aid in the understanding of the nature of the spawning site.

Objectives:

1.) Identify and characterize typical characteristics of a suspected razorback spawning site.

2.) Characterize habitat in the vicinity of the suspected spawning site.

3.) Identify other potential sites with similar characteristics

4.) Coordinate with the razorback sucker monitoring program to analyze findings.

Methods:

1.) Characterize spawning bar characteristics. As soon as the razorback sucker monitoring program identifies the presence of ripe fish at a location, a crew will be mobilized to the site to characterize the bar. (Up to three locations will be surveyed.) Topographic surveys will be completed for each of the sites identified utilizing total station or gps survey equipment and survey control bench marks established at each site.

At the same time, the structure of the bar will be assessed by completing point counts of the surface bed material (n=200 per sample or more) at each bar. Particles will be selected by the point count method over the full extent of the bar within the survey boundary. Size is determined by placing the rocks through a square hole in an aluminum plate, cut to represent an equivalent screen size from 1 cm through 10 cm at 1 cm increments, then 2 cm increments through 20 cm. Those larger than 20 cm are recorded as greater than 20 cm. Interstitial material smaller than 1 cm is not recorded.

Depth of open interstitial space (depth to embeddedness) will be measured on a 5 or 10-ft grid over the extend of the bar. Measurement will be made by working a hand between rocks until the fingers touch the sand embedded depth. The depth of penetration below the average top of cobble immediately adjacent to the sample point will be measured and recorded as the depth of open interstitial space.

Bar morphology will be determined by producing three-dimensional plots of the surveyed surface. Characteristics of the bar will be compared to other bars characterized during the 7-year research period.

The size distribution of cobble at each bar is computed and the D_{16}, D_{50} and D_{84} sizes reported and compared to previous years. Depth of open interstitial space will be computed as actual depth and multiples of mean cobble diameter.

Gross water quality parameters (temperature, DO, Ph, Conductivity) will be collected at the site and from local tributaries.

2.) Map habitat in the vicinity. Utilizing existing aerial photography taken near the flowrate at sampling as a base map, detailed habitat mapping will be completed to the long-term monitoring protocol for one mile up and downstream of the site. The information will be digitized and the data summarized.

3.) Identify other potential sites. Based on the characteristics identified at the suspected spawning sites, including vicinity habitat mapping, a review of mapped habitat will be completed and similar sites identified. A field investigation will be completed to characterize those identified as being similar utilizing the protocol in Task 1. This activity will be completed in the second year of the study based on preliminary data review in year one. The budget shown assumes complete surveys on 5 additional sites. If more sites are identified, the budget will be adjusted accordingly.

4.) Coordination with razorback sucker monitoring team. Data analysis will be coordinated with the razorback sucker monitoring team to compare habitat and substrate data with observed fish position. As disturbance of the fish at spawning time should be minimized, no field work will be completed without the approval of the Biology Committee and the razorback sucker monitoring team.

Products:

A summary report will be prepared covering the findings and comparing them to literature results, including unpublished observation data in the upper Colorado River Basin. A draft monitoring plan will also be prepared to track changes at this site and identify other potential sites.

Budget FY-2002:

Category	Staff-Days	Cost
Personnel:		
Field data collection and analysis	42	$ 20,956
Travel/per diem:	22	$ 3,350
Equipment Rental (boats, survey inst.)		$ 1,500
Misc. Supplies		$ 200
Overhead (10% of subcontract)		$ 1,319
Grand Total		**$ 27,325**

Budget FY-2003 (subject to change based on 2002 results):

Category	Staff-Days	Cost
Personnel: Field data collection and analysis	60	$ 31,066
Travel/per diem:	34	$ 4,350
Equipment Rental (boats, survey inst.)		$ 2,000
Misc. Supplies		$ 200
Overhead (10% of subcontract)		$ 1,300
Total		**$ 38,916**

Water Temperature Analysis - San Juan River
Fiscal Year 2002 Project Proposal

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Principal Investigator: Vince Lamarra
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Study Area:

The study area for this task is for the San Juan below Navajo Dam.

Collections:

None.

Background:

Water temperature monitoring in the San Juan River since 1992 has indicated a suppression of water temperature in the river due to hypolimnetic releases from Navajo Dam and mimicry of a natural hydrograph. The Program Evaluation Report identifies this temperature suppression as a potential limiting factor in the ability of Colorado pikeminnow to spawn at an appropriate time above RM 150.

Objective:

1.) Analyze collected and historical temperature data and define impact of hypolimnetic release on downstream water temperature.

2.) Assess the impact of the observed temperature change on range limitation and spawning date for Colorado Pikeminnow, razorback sucker and other species.

Methods:

1.) Analyze temperature data and define impact of hypolimnetic release on downstream water temperature. Temperature data collected since 1992 will be analyzed to develop a temperature/flow relationship for the San Juan River. This relationship will be used to assess expected temperatures form Navajo Dam to Bluff, Utah for the typical range of flow conditions experienced during the test period. The results will be compared to historically available data to assess the impact of reservoir operation on temperature.

2.) Assess the impact of the predicted water temperatures on range limitation and spawning date for Colorado pikeminnow, razorback sucker and other species. A literature search will be completed and the results summarized relative to the impact of water temperature on Colorado pikeminnow, razorback sucker and other species behavior. Utilizing the results of the temperature analysis, anticipated impacts to range limitation and spawning date by location will be assessed in the San Juan River.

Products:

A draft report documenting the temperature analysis and identifying relationships and temperature changes in the river relative to pre-dam conditions will be prepared by July 31, 2003, and a final report will be completed by September 30, 2003. A discussion paper will be produced summarizing the literature on the influence of water temperature on Colorado Pikeminnow behavior and assessing the impacts of water temperature in the San Juan.

Budget FY-2002:

Category	Staff-Days	Cost
Personnel:		
Data analysis & report	27	$ 18,040
Travel/per diem:	0	$ 0
Misc. supplies, copies,etc.		$ 500
Overhead		$ 700
Grand Total		**$ 19,240**

Navajo And San Juan River Temperature Model
Fiscal Year 2002 Project Proposal

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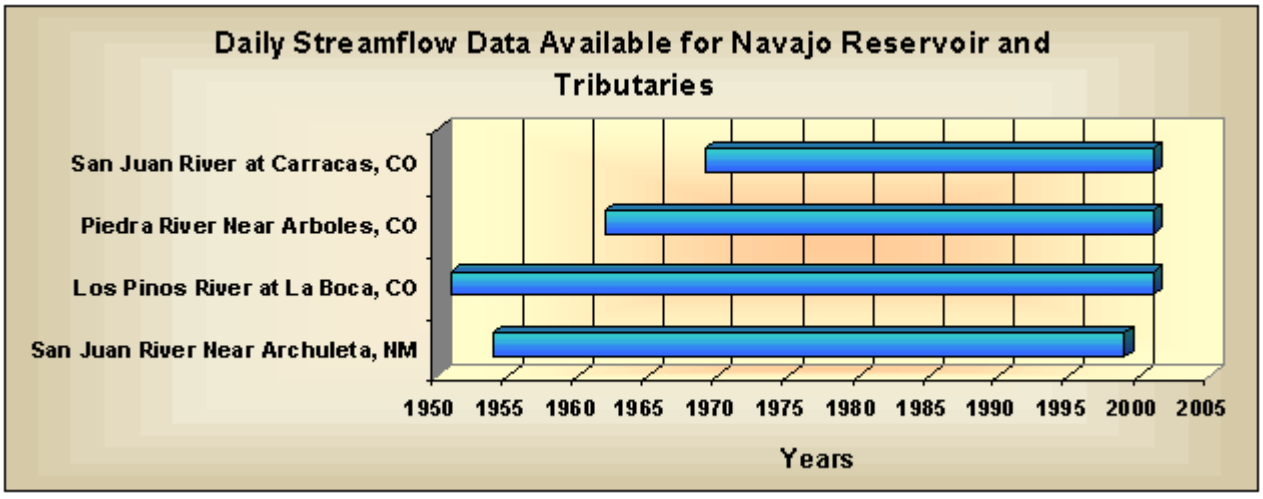
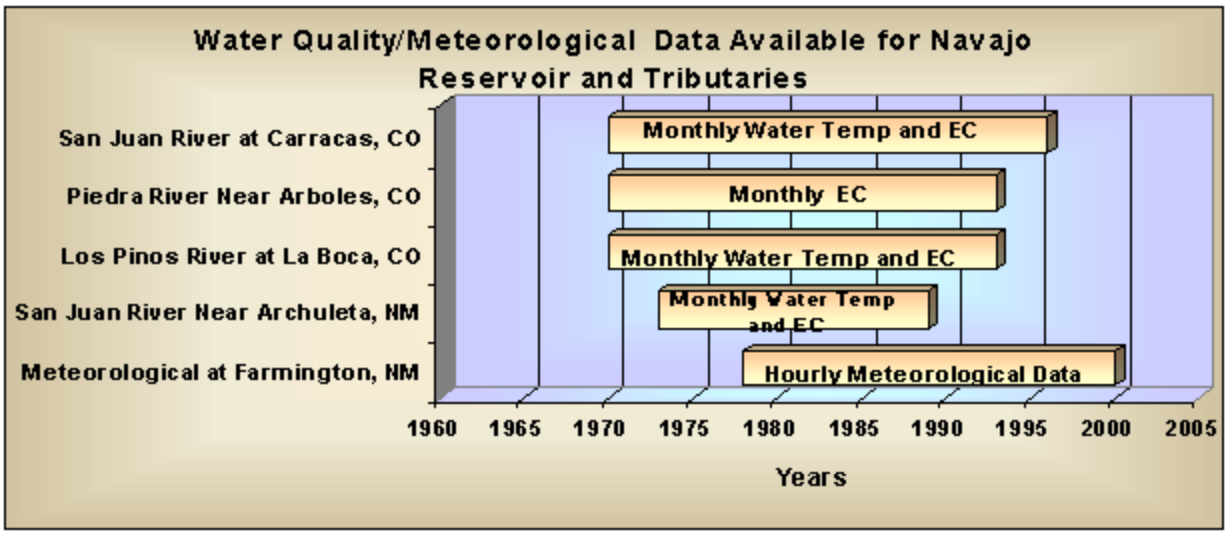
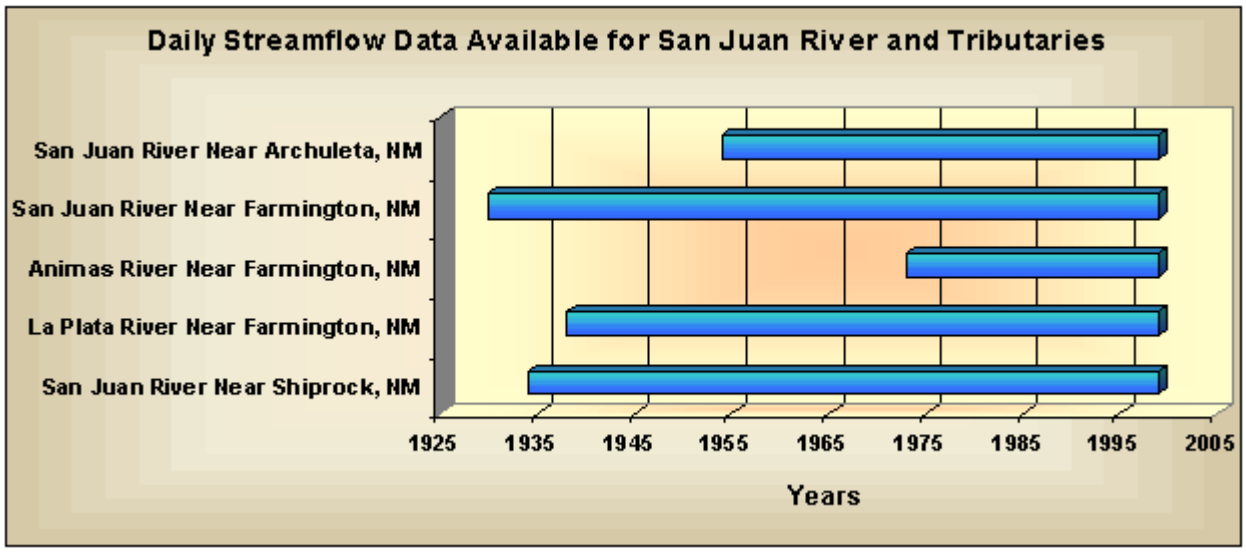
Background:

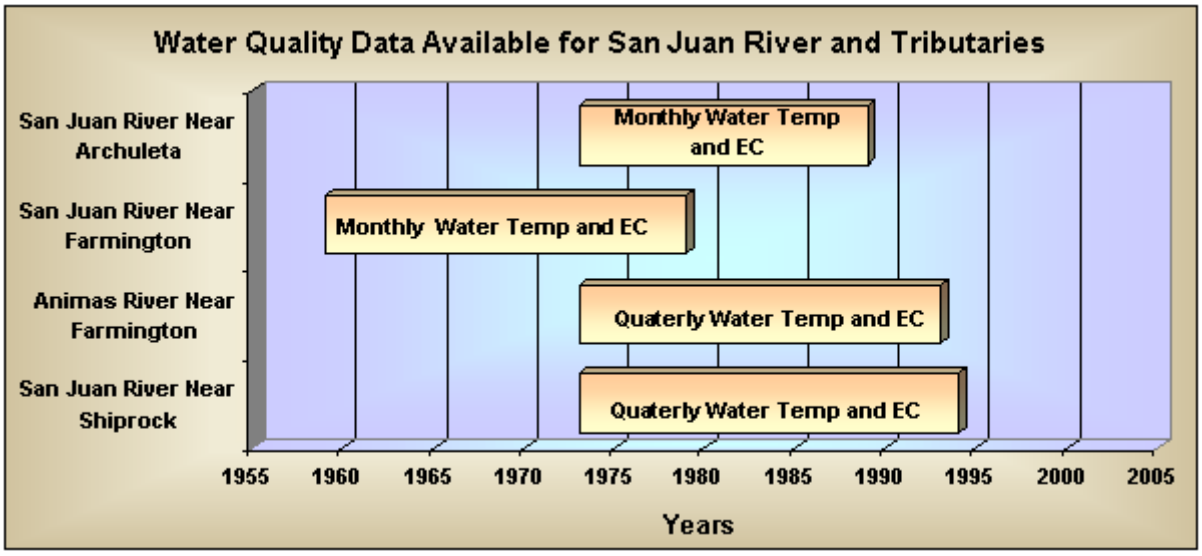
Studies have postulated that the changes in hydrology and water temperature releases from the Navajo Dam could benefit the Colorado pikeminnow and razorback sucker. It is believed that increasing temperatures in the San Juan River could benefit razorback sucker and Colorado pikeminnow growth and reproduction above River Mile 150 (near Shiprock NM). Specific temperature targets for the San Juan River are proposed to be developed in a separate scope of work for 2002. This scope of work proposes to use temperature models to determine the feasibility of achieving the desired river temperatures.

Presently there are streamflow, water quality and meteorological data available for the Navajo Reservoir and the San Juan River below the dam. The four graphs (below) indicate availability of data for certain periods from the USGS, Storet and National Weather Services Databases. Fundamentally the information available is sufficient to run temperature models. Based on the results of the models, more data could be gathered if fine-tuning is deemed necessary.




Water Quality/Meteorological Data Available for Navajo Reservoir and Tributaries
San Juan River at Carracas, CO
Piedra River Near Arboles, CO
Los Pinos River at La Boca, CO
San Juan River Near Archuleta, NM
Meteorological at Farmington, NM
Monthly Water Temp and EC
Monthly EC
Monthly Water Temp and EC
Monthly Water Temp and EC
Hourly Meteorological Data
1960
1965
1970
1975
1980
1985
1990
1995
2000
2005
Years


Daily Streamflow Data Available for San Juan River and Tributaries
San Juan River Near Archuleta, NM
San Juan River Near Farmington, NM
Animas River Near Farmington, NM
La Plata River Near Farmington, NM
San Juan River Near Shiprock, NM
1925
1935
1945
1955
1965
1975
1985
1995
2005
Years



Objectives:

The objectives for this study are to find the best solutions to the followings:

1.) To determine potential temperature regimes in the San Juan River down to approximately River Mile 150 that would result from various TCD options.

2.) Compare and contrast release water temperature for a historic period with and without TCD modifications.

3.) Determine the times of year in which water temperature targets can be met.

4.) Determine the effect of TCD on the heat budgets in the reservoir.

The above goals will be addressed through the utilization of reservoir and river temperature models applied to the Navajo Reservoir and the San Juan River using CE-QUAL-W2 (W2) and QUAL2E. These models will predict release temperatures with different TCD scenarios, and river temperature variations with changes in flow.

Study Area:

The study area includes Navajo Reservoir and the San Juan River below Navajo Dam to Shiprock.

Methods:

A two phased approach will be used to determine potential temperature regimes in the San Juan River below Navajo Dam to Shiprock. The W2 model will be used to determine available temperatures for various reservoir release options and to model the initial tailwater reach as mixing occurs if various penstock/TCD release options are utilized. The Qual2E model will use the output generated by the W2 model and flow information to determine downstream warming in the river to Shiprock. Phase one will define the best time period to incorporate the model of the Navajo Reservoir using W2. The W2 model requires meteorological data including hourly air temperatures, dew-point temperatures, wind speeds, wind directions and cloud covers, as well as data indicating daily inflows and outflows of all major tributaries, inflow and outflow water temperatures, total dissolved solids (TDS) and total suspended solids (TSS). Finally, detail geometry of the reservoir and the downstream tailwater must be obtained. A combination of topographical map and the "Instream Flow Incremental Methodology" study will be used to generate reservoir and river geometries for the Navajo system. The models will be calibrated using in-reservoir temperature profiles and release time-series water temperature data. The calibrated water temperature model will be used to test combinations of flows and withdraw levels in the reservoir to meet the target temperature range for the downstream needs of the San Juan River. Similar work has been done on the Glen Canyon Dam where a temperature control device (TCD) is being considered to help endangered fishes. We applied W2 on Lake Powell. We were able to determine the earliest date when the temperature target can be met, whether the heat budget in the reservoir is affected, and the most effective TCD design. Phase two will incorporate reservoir release water temperatures modeled by W2 as inputs to the river model (QUAL2E).

Schedule:

1.) Analyze data to select best period for modeling (May 2002).

2.) Build detail geometry data for Navajo Reservoir and downstream tailwater (July 2002).

3.) Input model data (September 2002).

4.) Calibrate reservoir temperature models (January 2003).

5.) Analyze temperature scenarios (March 2003).

6.) San Juan River temperature model using QUAL2E (May 2003).

7.) Report (September 2003).

Deliverables/Due Dates:

1.) Data analysis: May 2002

2.) Detail Geometry of reservoir: July 2002

3.) Input model data: September 2002

Budget FY-2002:

Labor	$ 21,720.00
Travel	$ 1,240.00
TOTAL	**$ 22,960.00**

FY - 2003:

Deliverables/Due Dates:

1.) Reservoir model calibrations: January 2003

2.) Scenario analyses: March 2003

3.) River model: May 2003

4.) Final report: September 2003

Budget FY-2003:

Labor	$ 22,400.00
Travel	$ 1,240.00
TOTAL	**$ 23,640.00**

Budget Summary (FY 2002 - 2003):

FY-2002	$ 22,960.00
FY-2003	$ 23,640.00
TOTAL	**$ 46,600.00**

References:

Cole, T. M. 1995. "A Two-Dimensional, Laterally Averaged, Hydrodynamic and Water Quality Model, Version 2.0", Final Report E-86-5, U.S. Army Corps of Engineers.

Holden, B. P. (Ed.). 1999. "Flow Recommendations for San Juan River", San Juan River Basin Recovery Implementation Program, U.S. Fish and Wildlife Service, Albuquerque, NM.

Temperature Modeling for Navajo Reservoir and San

	Temperature Modeling for FY-2002		
Budget Category	**Unit (day)**	**Unit Cost**	**Total Cost**
Personnel			
WQ scientist (UCRO)	5	$504.00	$2,520.00
Engineer (UCRO)	30	$360.00	$10,800.00
Technician (UCRO)	10	$280.00	$2,800.00
Grad Student (BYU)	20	$280.00	$5,600.00
Total Personnel Costs			$21,720.00
Travel	**Unit (person)**	**Unit Cost**	**Total Cost**
Airfare	4	$200.00	$800.00
Per diem	4	$110.00	$440.00
Total Travel Cost			$1,240.00
Total Cost for FY-2002			**$22,960.00**

	Temperature Modeling for FY-2003		
Budget Category	**Unit (day)**	**Unit Cost**	**Total Cost**
Personnel			
*Model Review	10	$800.00	$8,000.00
Engineer (UCRO)	40	$360.00	$14,400.00
Total Personnel Costs			$22,400.00
Travel	**Unit (person)**	**Unit Cost**	**Total Cost**
Airfare	4	$200.00	$800.00
Per diem	4	$110.00	$440.00
Total Travel Cost			$1,240.00
Total Cost for FY-2003			**$23,640.00**

Total Project Cost	**$46,600.00**

*Model Review: The models will be reviewed by J. E. Edinger Associates, Inc. Research and Applications in Watershed and Waterbody Science Consultants

Determination of Occurrence of Hybridization of San Juan River Razorback Sucker Through Genetic Screening of Larval Fishes
Fiscal Year 2002 Project Proposal

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Background:

The first record of naturally reproducing San Juan River razorback sucker was on 21-22 May 1998 with the collection of two larval fish near river miles 88.8 and 80.2. These specimens provide verification of spawning by the augmented-introduced population. In 1999, seven larval razorback sucker were collected between 4 May and 14 June. The seven larvae (razorback) were taken in backwater or low velocity habitats located between river miles 96.2 and 11.5 with almost half (n=3) of those individuals being taken downstream of Mexican Hat. The considerable increase in number of razorback sucker collected between 1999 and 2000 was deemed indicative of the gradually increasing number of adult razorback sucker being recruited into the spawning cohort. Tentative identifications of larval fish collected during the 2000 survey revealed 138 larval razorback sucker in 24 separate collections. The putative larval razorback sucker were collected in low velocity habitats between river miles 124.8 and 8.1 with the lowest-most sampling location (that yielded larval razorback sucker; RM 8.1) producing over 85 individuals in a single sample (26 May 2000). Conversely, the uppermost collection of larval razorback sucker was less than four river miles downstream of the upper boundary of the study area on 1 June 2000.

The results of this investigation suggest continually increasing recruitment of adult reproductively mature razorback sucker to the population. Since 1998, there has been a logarithmic increase in the number of individuals collected and we expect this trend to continue in 2001. The number of larval razorback sucker being spawned, while increasing annually, is still not deemed of sufficient magnitude to allow for recruitment of larvae to the juvenile population. The small-bodied fish collecting effort undertaken during the fall monitoring program (conducted by NMGF) is the sampling mechanism that will be used to determine if recruitment between these developmental stages has occurred.

Hybridization of razorback sucker with sympatric sucker taxa occurs throughout the Colorado River Basin. Lower Colorado River Basin razorback sucker x flannelmouth sucker hybrids have been reported in both Lake Mohave (Minckley 1983, Bozek et al. 1984) and the Grand Canyon reach of the Colorado River (Suttkus and Clemmer 1979). There are numerous reports of razorback sucker x flannelmouth sucker hybrids in the Upper Colorado River Basin (Banks 1964, Holden and Stalnaker 1975, McAda and Wydoski 1980, Tyus and Karp 1990). Holden (1973) collected nearly as many hydrid razorback sucker x flannelmouth sucker (n=40) as putative pure

razorback sucker (n=53). He also reported (Holden 1973) that the incidence of hybridization in the Upper Basin appear to be increasing.

In their study of spawning and movement of razorback sucker in the Green River, Tyus and Karp (1990) reported capturing both ripe flannelmouth sucker and bluehead sucker syntopically with ripe razorback sucker. They also documented suspected flannelmouth sucker x razorback sucker hybrids (n=17) within an aggregation of ripe flannelmouth sucker, bluehead sucker, and razorback sucker in Yampa Canyon and the upper Green River (Tyus and Karp 1990). These and the aforementioned hybrids were determined based on morphological characters that appeared intermediate when compared with putative pure taxa.

Given the limited number of reproductively active razorback sucker in the San Juan River and small number of razorback sucker larvae being collected it was not previously deemed necessary to perform genetic analysis to ascertain the parental stock of larval San Juan River razorback sucker (i.e., whether they were intergeneric hybrids). With the collection of 138 larval razorback sucker in 2000 and the anticipated collection of an equal or greater number of razorback sucker larvae in 2001, reproduction by this species has achieved a level (as determined by the number of individuals being collected) sufficient to warrant such an investigation.

Hybridization has been identified as an important factor that influences survivorship and successful recruitment to the breeding population (Pitts et al. 1997). Hybrid offspring often have higher probabilities of early mortality (Dowling and Moore 1985, Pitts et al. 1997). The persistence of hybrid offspring may facilitate genetic exchange among species disrupting the genetic cohesion of species. Hybridization is known to occur most frequently when environmental conditions have changed dramatically from historical conditions. Given the changes in the San Juan River system, the absence of resident population, and the recent introduction of razorback sucker, the opportunity for hybridization of this species may be greater than documented in other Colorado River Basin systems.

To achieve recovery of this species, it is essential to develop baseline genetic information that will document whether hybridization among re-established razorback sucker, flannelmouth sucker, and bluehead sucker is occurring. This objective was identified in the San Juan River Basin Recovery Implementation Program Plan Item (5.3.7 and 5.3.7.1). These objectives are designed to characterize the genetic makeup of endangered fish species with the goal of maintaining the genetic diversity of these taxa.

In contrast to many other Upper (and Lower) Colorado River Basin river drainages, the San Juan River did not have a resident population of razorback sucker at the time that augmentation of this species was initiated. The presumption has been that individuals repatriated to reaches containing resident populations of razorback sucker would intermingle and spawn with resident razorback sucker. The absence of adult razorback sucker from the San Juan River when augmentation was initiated was a concern because of the increased possibility that augmented razorback sucker would spawn with other catostomids. The number of flannelmouth sucker and bluehead sucker present in the San Juan River as compared to the number of released razorback sucker (a maximum of about 4,000) reduced the probability of razorback sucker pairing versus

hybridization. The former statement is a probability-based presumption and does not take into consideration premating mechanisms.

The 1998-2000 larval razorback sucker study resulted in the first records of naturally reproducing population of augmented razorback sucker. Since it is likely that the augmentation process will be duplicated in other portions of this species former range, it is important to determine if these individuals are the progeny of razorback sucker parentage or are hybrids. This information is most effectively obtained from focused genetic analysis.

A number of genetic screening techniques, used routinely on fish larvae in our laboratory, have been developed and are especially well suited to investigate hybridization. This goal will be accomplished through the use of a DNA based screening of larval fishes that will unambiguously identify parentage of larval individuals. Details regarding the genetic techniques to be employed are appended to this study proposal.

Study Area:

The principal sampling area for this study will be the San Juan River between Cudei Diversion Dam (near RM 142) and the Clay Hills boat landing (ca. RM 5) just above Lake Powell in Utah. A spring 2000 collection of larval razorback sucker at RM 124.8 indicated the need to expand the upstream boundary of the study area (formerly RM 128). Beginning in FY 2002, sampling will include an additional 14 river miles of the San Juan River (the reach between Cudei Diversion Dam and RM 128). As in all post 1999 sampling efforts, the study will include making collections in reaches of the San Juan River under the jurisdiction of the National Park Service. All collections used in this genetic study will be made as part of the San Juan River larval razorback sucker survey.

Objectives:

1.) Develop baseline information based solely on FY 2002 collections that documents the presence or absence of hybridization among re-established razorback sucker, flannelmouth sucker, and bluehead sucker.

2.) Provide unambiguous identification of parentage of larval suckers collected in the San Juan River during FY 2002.

3.) Provide spatial and temporal delineation of parentage of larval suckers collected in the San Juan River during FY 2002.

Methods:

Larval razorback sucker that will be genetically examined in this study proposal will be obtained under the "San Juan River Larval Razorback Sucker Survey" submitted by Gottlieb, Brandenburg, and Platania. The protocol for acquisition of those collections is provided in the aforementioned study proposal and is also appended to this proposal. There are no costs included in this proposal associated with the collection of the larval razorback sucker as all

collection costs are being borne by the "San Juan River Larval Razorback Sucker Survey Fiscal Year 2002 Project Proposal."

The only change in sampling methodology and protocol for the San Juan River Larval Razorback Sucker Survey that would need to be initiated, if this genetic study proposal is approved, would be preservation of samples in ethanol instead of buffered formalin (as is currently standard procedure).

Products:

A draft report for the 2002 razorback sucker sampling activities will be prepared and distributed to the San Juan River Biology Committee for review by 31 March 2003. Upon receipt of written comments, that report will be finalized and disseminated to members of the San Juan River Biology Committee 1 June 2003. Fish collected from this study will be curated in the Division of Fishes, Museum of Southwestern Biology (MSB), Department of Biology, at the University of New Mexico. Original field notes will be retained in the Division of Fishes and collection information will be electronically stored in a permanent MSB database program. Electronic copies of the field and collection data will be transferred to the San Juan River database manager following the successful protocol previously employed.

Budget FY-2002:

Personnel			
	Laboratory Research (16 man-days) (perform genetic analysis)	$	8,000
Travel and per diem			
	Non-Field per diem (SJR meeting attendance)	$	500
Equipment and Supplies			
	8 reactions x 100 individuals @ $5.00/reaction	$	4,000
	Total	$	12,500
	Administrative Overhead	$	1,875
GRAND TOTAL		$	**14,375**

There is no outyear funding for this project.

Literature Cited:

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APPENDIX I – Molecular Methods

Total genomic DNA will be isolated from individual putative larval razorback sucker. Larvae are suspended in 200 ml STE buffer (0.1 M NaCl, 0.05 M Tris-HCl pH 7.5, 0.001 M EDTA), 10 ml of a 20% solution of SDS, and 4 ml proteinase K (10 mg/ml stock). Samples are then incubated at 55^{o}C from four hours to overnight in a circulating water bath. Digested samples will be extracted with 200-ml phenol-chloroform-isoamyl alcohol (25:24:1) and then extracted once with 200-ml chloroform. Finally, DNA is precipitated by adding 2.5 volumes of ice cold 100% EtOH and 0.1 vol of 2 M NaCl, and cooling at -80^{o}C for 15 minutes. After precipitation and centrifugation, DNA pellets are dried in a vacuum centrifuge, and resuspended in 30 ml of sterile water.
Individual larvae will be screened using DNA based techniques that rely on amplifying DNA

fragments via polymerase chain reaction (PCR). This technique is well suited for screening larval fishes because very little sample DNA is needed for screening. Two kinds of genetic markers have been developed in our laboratory for use in studying hybridization. The first class of markers, DNA microsatellites, are short nuclear elements that are highly variable within and among species. Because microsatellite markers are found in the nuclear genome, genetic profiles of offspring are inherited both from their mother and father. It is impossible to determine based solely on microsatellite DNA, which species was which parent. In many cases, fish hybridization is unidirectional, *i.e.*; one species is always the female parent of hybrid offspring. Understanding the directionality of hybridization is critical for many management applications, and thus we propose to use a strictly maternally inherited genetic marker to identify the female parent of putative hybrids. The markers we have developed for this purpose are in the mitochondrial (mt) genome and are inherited strictly maternally. Thus, the mtDNA profile unambiguously identifies the female parent in a hybridization event.

Eight microsatellite loci have been developed for the razorback sucker and are used routinely in our laboratory on a number of catostomid species. Microsatellites are found in the genomes of many vertebrates, including fish. They are a DNA-based marker that is analyzed via polymerase chain reaction (PCR), so they can be applied to very small ethanol preserved specimens, such as fish larvae (Turner, 2001). Numerous microsatellite loci originally identified for razorback sucker work well for other catostomids. More importantly, these loci exhibit fixed genetic differences (e.g., they are species-diagnostic) between the sucker species tested in our laboratory (white sucker and Rio Grande sucker). Other laboratories have demonstrated fixed genetic differences in these loci between razorback sucker, bluehead sucker, and desert sucker (T. Dowling, Arizona State University, personal communication). The demonstration of fixed genetic differences indicates that these markers are especially well suited for hybridization studies of San Juan River catostomids

Comparative mtDNA sequence data from several sucker species has been used, in our laboratory, to identify polymorphic 300 bp (approximate) fragments for screening. PCR primers were designed to permit screening using single-stranded conformational polymorphisms (SSCPs) following methods outlined below. Total genomic DNA will be isolated from fin clips of adult individuals and entire larval fishes. PCR amplification will be conducted in 10-ml volumes in an Omn-E thermal cycler (Hybaid). Microsatellite loci are analyzed on 6% polyacrylamide gels (Sequagel, National Diagnostics) and are currently visualized by autoradiography. They will ultimately be optimized for the ABI 377. Alleles will be scored by length in base pairs relative to the known size standards. For mtDNA, sample DNA will be subjected to PCR using primer pairs described above. Following PCR, samples will be denatured in boiling water for 5 minutes, and immediately quenched in ice slurry to prevent renaturation of double-stranded DNA. Cold samples will be loaded into a 5% non-denaturing polyacrylamide (37.5:1 acrylamide:bis-acrylimade) gel with 5% glycerol. The samples will be subjected to electrophoresis at 10 W for 16 h at room temperature. Fragments will be visualized by autoradiography and scored by comparing their relative mobilities. At least two representives of variable haplotypes on each gel will be sequenced using an ABI 377 automated sequencing apparatus. All of the equipment necessary for successful completion of the project is present in our laboratory.

Turner, T. F. 2001. Comparative study of larval transport and gene flow in darters. Copeia 2001(3): 766-774.

APPENDIX II:

Collection Methods: (modified from San Juan River Larval Razorback Sucker Survey Fiscal Year 2002 Project Proposal)

Sampling for razorback sucker larvae will be conducted in the San Juan River between Cudei (RM 142) and Clay Hills (RM 2.9) from early-April through early June using sampling techniques that will provide sufficient number of individual fish necessary to meet study objectives. GPS readings will be taken at each sampling locality, and researchers will record UTM coordinates and zone corresponding with each field number as agreed upon at the May, 2001 meeting of the San Juan River Biological Committee. Access to the river shall be acquired through the use of inflatable rafts. The tentative sampling schedule will be on a bi-weekly (approximately) interval.

Sampling efforts for larval fish will be concentrated in low velocity habitats. Samples in those habitats will be collected with small mesh seines and light-traps. Habitat type, length, maximum depth and substrate of the habitat will be recorded. For seine samples, length and number of each seine haul will be determined. Specimens will be preserved in the field (in 95% ethanol) for future laboratory processing. Larval fish tentatively identified as razorback sucker will be sent to Darrel E. Snyder (Larval Fish Laboratory, Colorado State University) for verification of identification. Upon confirmation and return of those specimens, a subsample of larval razorback sucker will be selected for genetic analysis. Those sub-samples will contain (minimally) larval razorback sucker that represent the temporal (earliest and latest collections of this taxon) and spatial (most upstream and downstream collections of this taxon) extremes recorded during the 2002 San Juan River Larval Razorback Sucker Survey.